Effects of Diet, Ginger Root Oil, and Elevation on the Mating Competitiveness of Male Mediterranean Fruit Flies (Diptera: Tephritidae) from a Mass-Reared, Genetic Sexing Strain in Guatemala

TODD E. SHELLY,[1] PEDRO RENDON,[2] EMILIO HERNANDEZ,[3] SERGIO SALGADO,[3] DONALD MCINNIS,[4] ETHEL VILLALOBOS,[5] AND PABLO LIEDO[3]

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ABSTRACT The release of sterile males is a key component of an areawide program to eradicate the Mediterranean fruit fly, *Ceratitis capitata* (Wiedemann), from Guatemala and southern Mexico. The objective of our study was to assess the effects of adult diet, exposure to ginger root oil (*Zingiber officinale* Roscoe), and elevation on the mating competitiveness of the sterile males used in an areawide program. Sterile males were maintained on a protein-sugar (protein-fed) or a sugar-only (protein-deprived) diet and were exposed (for 4 h 1 d before testing) or not exposed to ginger root oil. In field-cage trials conducted at a high (1,500 m) and low (700 m) site, we monitored the influence of these treatments on the mating success of sterile males in competition with wild males (reared exclusively on the protein-sugar diet and without ginger root oil exposure) for wild females. Elevation and ginger root oil exposure had significant effects, with sterile males having higher mating success at the low-elevation site and ginger root oil-exposed males having greater success than ginger root oil-deprived males at both sites. Diet did not have a significant overall effect, and its influence varied with elevation (dietary protein seemed to provide an advantage at the high-elevation site but not at the low-elevation site). Possible implications of these findings for eradication programs against the Mediterranean fruit fly are discussed.

KEY WORDS *Ceratitis capitata*, sterile insect technique, mating competitiveness

STERILE INSECT TECHNIQUE (SIT) is an environmentally benign method used widely in controlling infestations of agriculturally important tephritid fruit flies, particularly the Mediterranean fruit fly, *Ceratitis capitata* (Wiedemann) (Hendrichs et al. 2002). Sterile insect technique involves the production and irradiation (sterilization) of large numbers of individuals of the target pest species, release of sterile individuals into the environment, and the reduction of the reproductive potential of the wild population through matings between wild females and sterile males (which yield infertile embryos). Because the success of SIT depends on the ability of sterile males to copulate with wild females, it is essential that the mass-rearing protocol itself does not produce males with diminished mating competitiveness (Robinson et al. 2002).

Unfortunately, the mass-rearing procedures inherent to SIT often lead to a reduction in the mating competitiveness and viability of released *C. capitata* males, particularly in long-established strains (Cayol 2000, Lance et al. 2000). The deterioration of *C. capitata* strains results from a combination of factors, including genetic drift with its concomitant loss of genetic variability and intense artificial selection imposed by laboratory conditions (Leppla and Ozaki 1991). Because of these problems, sterile males typically have low mating success relative to wild males (Cayol 2000, Robinson et al. 2002).

Thus, a persistent and important challenge for SIT is the development of simple and inexpensive means to enhance the mating performance of released, sterile *C. capitata* males in the wild. Recent research suggests two possible approaches to accomplish this goal. Studies by Yuval et al. (2002, and references therein) demonstrated that, for mass-reared male Mediterranean fruit flies, the addition of protein to the adult diet increased signaling (pheromone-calling) activity and mating success relative to males that were fed only sugar as adults. They also found that females mated to protein-fed males were less likely to remate than females first mated to protein-deprived males. In addition, Shelly et al. (2002a and references therein) have

[1] USDA–APHIS, P.O. Box 1040, Waimanalo, HI 96795, and Hawaiian Evolutionary Biology Program, University of Hawaii, Honolulu, HI 96822.
[2] USDA–APHIS, Methods Development Station, c/o American Embassy, Guatemala City, APO AA 34024–3319.
[3] El Colegio de la Frontera Sur, Apartado Postal 36, 30700, Tapachula, Chiapas, Mexico.
[4] USDA–ARS, 2727 Woodlawn Drive, Honolulu, HI 96822.
[5] Department of Plant and Environmental Protection Services, University of Hawaii, Honolulu, HI 96822.

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shown that prerelease exposure to particular attractants increases the mating success of sterile male Mediterranean fruit flies in field cage studies. In particular, male exposure to ginger root oil (*Zingiber officinale* Roscoe), which contains the known male attractant α-copaene (Flath et al. 1994a,b), has a strong positive effect on the mating frequency of male Mediterranean fruit flies.

The objective of the current study was to assess whether the addition of protein to the adult diet and exposure to ginger root oil influenced the mating success of sterile males from the mass-reared strain presently used in the Mediterranean fruit fly eradication program in Guatemala and southern Mexico (Villasenor et al. 2000). In addition, given the large altitudinal range (sea level to 1,700 m) over which wild Mediterranean fruit flies occur in this region, we investigated whether the relative mating success of sterile males under different diet and ginger root oil treatments varied between two sites differing in elevation and ambient temperature.

Materials and Methods

Study Sites. Mating trials were conducted between February and April 2001 and 2002 at two locations in Guatemala. The high-elevation site (1,500 m) was a coffee plantation (San Augustin) located 15 km southeast of Guatemala City, and the low-elevation site (700 m) was an agricultural experiment station (Sabana Grande) of the Universidad de San Carlos located 60 km south of Guatemala City. At both sites, nylon-mesh field cages (3 m in diameter $\times$ 2.5 m in height) were placed over individual coffee plants (*Coffea arabica* L.) growing beneath an open canopy (10–15 m in height) of shade trees. The caged plants were of similar size (1.75–2.0 m in height) and, with few exceptions, lacked flowers or fruits. Air temperatures were recorded to the nearest 0.5°C at 30-min intervals during testing at shaded sites 1–1.5 m above ground in three to four cages per day (these readings were averaged to yield a single temperature per time interval per day).

Flies. Wild flies were reared from coffee fruits collected during January through March in the vicinity of Antigua (1,200–1,500 m). Fruits were returned to the laboratory, and the wild flies completed their development in situ and pupated in sawdust placed beneath the fruits. Emerging adults were separated by sex within 1 d of eclosion and were given water and a protein-sugar diet (yeast hydrolysate and sucrose in a 3:1 ratio by weight). Flies were held at 24–26°C and 65–75% RH and received artificial and natural light under a natural photoperiod of 12:12 (L:D) h. Wild flies were 9 d old when tested and were used for only one trial. Owing to a shortage of wild flies, "wild-like" flies from a stock reared on papaya (*Carica papaya* L.) for two generations were used in 16 of the 36 mating trials conducted at the low elevation site. These 16 trials were purposely distributed evenly among the four treatments examined (see below), i.e., for each treatment five trials were run using wild males and four trials were run using wild-like males. No difference in the relative mating success of wild and wild-like males was found for any of the treatments ($P > 0.05$ in all tests, Mann-Whitney test), and consequently data from wild and wild-like males were pooled.

Mass-reared flies (males) were obtained from the USDA–APHIS Mediterranean Fruit Fly rearing facility in El Pino from a *tsl* genetic sexing strain (Vienna 7/Tol 99), in which female eggs are selectively killed by exposure to high temperature to allow male-only releases (Franz et al. 1994). Pupae were obtained 2 d before eclosion after irradiation at 100 Gy with a Co^{60} irradiator. Upon emergence, *tsl* males were provided with water and either the protein-sugar mixture or sugar-only; males in these treatments were referred to as protein-fed and protein-deprived, respectively. The *tsl* males were held under the same conditions as wild males and were not subject to prolonged chilling before use (as done to load and hold mass-reared flies in release aircraft in SIT programs). The *tsl* males were 5 d old when tested (sexual maturity is attained at an earlier age in this strain than in wild flies; Lance et al. 2000) and were used for only one trial.

Mating Trials. Two experiments were conducted. In the first, we established a competitive mating environment in the field cages by releasing equal numbers of wild males, *tsl* males, and wild females. These trials were run at both study sites using *tsl* males that were (1) protein-fed or protein-deprived and (2) ginger root oil-exposed or ginger root oil-deprived (see below). In the second experiment, protein-fed *tsl* males that were ginger root oil-exposed or ginger root oil-deprived competed against one another (i.e., no wild males were used) for wild females in trials conducted at both study sites. We did not run parallel experiments for protein-deprived, *tsl* males. In both experiments, wild flies were fed the protein-sugar mixture, and in experiment 1 wild males were not exposed to ginger root oil.

The method for exposing *tsl* males to ginger root oil (Citrus and Allied Essences, Lake Success, NY) followed that described in Shelly (2001). The ginger root oil contained 0.4% α-copaene (T. W. Phillips, personal communication) along with other sesquiterpenes whose effect on *C. capitata* either independently or in combination with α-copaene remain largely unknown (but see Flath et al. 1994a, 1994b). Groups of 25 males were placed into plastic containers covered with nylon mesh (volume 1 liter). A small filter paper disk to which 20 μl of ginger root oil had been applied was suspended from the mesh into the cup. The disk was removed 4 h later, and males were supplied with water and the appropriate diet. Males were exposed to ginger root oil 1 d before testing. Exposure was conducted in a separate building from that used to hold all other flies, thus avoiding inadvertent exposure of wild flies or control *tsl* (ginger root oil-deprived) males.

Mating trials were conducted in a similar manner for both experiments. Males were released at $\approx$0715 hours. One group of males was marked 1 d before testing by cooling individuals and placing a small dot

of enamel paint on the thorax. This procedure had no adverse effects, and males resumed normal activities within minutes of handling. The type of male marked (wild or *tsl* in the first experiment and ginger root oil-exposed or ginger root oil-deprived *tsl* males in the second experiment) was alternated between successive test days. Females were released 15 min later than the males at both sites. Owing to variable availability of wild flies, we released either 100 females and 100 males of each type being tested (i.e., 300 flies total) or 50 females and 50 males of each type tested (i.e., 150 flies total). After female release, the field cages were monitored continuously until 1230 hours at the high site and 1200 hours at the low site, mating pairs were collected in vials, males were identified, and the time of collection (assumed to be equivalent with the start of copulation) was recorded. In experiment 1, vials containing mated pairs were kept in the shade and monitored frequently to determine the termination time of copulation. Mating trials were conducted on 11 and 10 d at the high- and low- elevation sites, respectively, and three to six field cages were run on a given day with one or two observers per cage.

During fieldwork at the high site, we noted an inverse relationship between copulation duration and the time at which copulation was initiated (i.e., early-starting copulations were longer than late-starting ones; see below). To determine whether this trend reflected size assortative mating (i.e., early copulations may involve larger flies, which require longer intervals for mating), we collected the first and last 15 pairs of wild-by-wild matings from three cages in experiment 1 at the high-elevation site and measured head widths of both sexes (using a Wild M8 stereomicroscope equipped with a disc micrometer). Male and female sizes were then compared between early- and late-mating groups for the individual cages.

Statistical Analyses. In experiment 1, the effects of elevation, ginger root oil exposure, and diet on mating competition were tested in a three-way analysis of variance (ANOVA) with the proportion of matings obtained by *tsl* males as the dependent variable. Additional pairwise comparisons of relative mating frequency were made using Student's *t*-test. In both the ANOVA and *t*-tests, proportions were transformed using the arcsine transformation to normalize the data. In experiment 2, the number of flies used per replicate was constant, and pairwise comparisons of mating frequency were made using the raw data in *t*-tests. Assumptions of normality (tested using the Kolmogorov-Smirnov distribution with the Lillefors correction) and homoscedasticity (tested using Levene's median test) were met in all analyses of mating success in both experiments. Distributions of start times for matings were compared using the χ^2 test. Analyses of copulation duration (data from experiment 1 only) were performed using ANOVA or *t*-tests with $\log_{10}$ transformed values. Although the assumption of normality was not always met, we nonetheless used ANOVA, because even large deviations from normality have little effect on its validity, particularly, as in the present example, when sample sizes are large (Zar 1996).

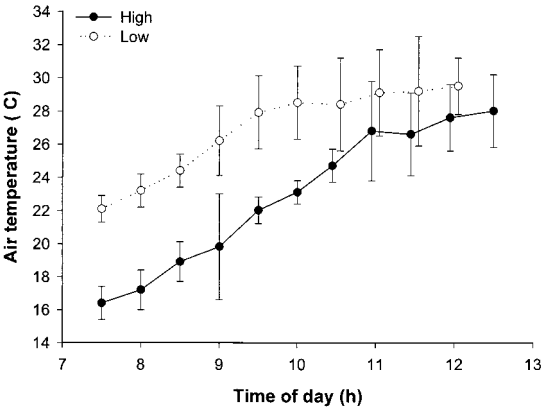

Fig. 1. Air temperatures recorded at 30-min intervals during mating trials at the high- and low-elevation sites. Values represent means (±1 SD) over 11 d at the high-elevation site and 10 d at the low-elevation site.

Results

Site Differences in Air Temperature. The temporal pattern of temperature increase differed between the two sites (Fig. 1). At the high-elevation site, air temperature increased monotonically throughout the morning from ≈16°C at 0730 hours to 29°C at 1230 hours. In contrast, air temperature at the low-elevation site rose from 22°C at 0730 hours to 28°C at 0930 hours but then increased only slightly over the rest of the morning. For all 30-min intervals, air temperature was, on average, significantly higher at the low- than at the high-elevation site ($P < 0.05$ and df = 19 for all comparisons; *t*-test). However, the magnitude of the temperature difference between sites (using average values at 30-min intervals) decreased significantly over the course of the morning ($r = -0.87$; $P < 0.01$; $n = 10$ intervals between 0730 and 1200 hours).

Experiment 1: Competition between *tsl* and Wild Males. The raw data for this experiment are summarized in Table 1, and the relative mating success of *tsl* males under the different treatments is presented in Fig. 2. In direct competition with wild males, the mating success of *tsl* males varied significantly with elevation and ginger root oil exposure but not with diet (Table 2). However, a significant interaction was detected between elevation and diet, indicating that dietary effect was dependent on the elevation. Interactions between elevation and ginger root oil exposure, diet and ginger root oil exposure, or all three factors were not significant.

Elevation had a dramatic effect on the mating tests, with the *tsl* males competing more successfully at the lower site. Over the four combinations of diet and ginger root oil exposure tested at each site, *tsl* males obtained, on average, 42–68% (mean = 54.2%, $n = 36$) of all matings at the low-elevation site compared with only 10–41% (mean = 25.2%, $n = 36$) at the high-

Table 1. Results of field-cage tests that examined the effects of elevation, adult diet, and exposure to ginger root oil (GRO) on the mating performance of *tsl* versus wild males (experiment 1)

	No. matings	
	tsl males	Wild males
	High-elevation site	
Protein/sugar diet		
No GRO exposure (100)	12.1 (6.5)	50.4 (5.8)
GRO exposure (100)	28.3 (11.3)	40.5 (12.8)
Sugar only diet		
No GRO exposure (50)	3.6 (3.2)	28.9 (4.4)
GRO exposure (50)	10.4 (3.2)	23.4 (4.0)
	Low-elevation site	
Protein/sugar diet		
No GRO exposure (50)	12.8 (4.0)	17.2 (3.1)
GRO exposure (50)	19.9 (4.0)	13.0 (5.6)
Sugar-only diet		
No GRO exposure (50)	14.5 (3.7)	16.5 (4.7)
GRO exposure (50)	22.7 (4.1)	11.1 (5.0)

Mean number (± 1 SD) of matings per replicate (field cage) are presented. Nine replicates were conducted for all treatments. The values 50 or 100 refer to the numbers of females and males of each type used per replicate.

elevation site. Ginger root oil exposure also had a significant positive effect on the mating success of *tsl* males. At the high-elevation site, ginger root oil exposure increased the relative mating success of *tsl* males 2.1 and 3.0 times over the ginger root oil-deprived, protein-fed ($t = 3.7$, df = 16 in this and the following tests; $P < 0.01$) and protein-deprived ($t = 5.3$, $P < 0.001$) males, respectively. Similarly, at the low-elevation site, ginger root oil exposure increased the relative mating success of *tsl* males 1.4 times over both the ginger root oil-deprived, protein-fed ($t = 3.3$, $P < 0.01$) and the protein-deprived ($t = 4.1$, $P < 0.001$), males respectively.

As indicated by the ANOVA, the effect of diet differed with elevation. For a given ginger root oil treatment, the protein-fed males, on average, obtained more matings than the protein-deprived males at the high-elevation site, whereas the opposite was true at the low-elevation site (Fig. 2). At the high-elevation site, the difference in relative mating success between protein-fed and protein-deprived males was statistically significant for ginger root oil-deprived males ($t = 2.4$, df = 16 in this and the following tests, $P < 0.05$) but not for ginger root oil-exposed males ($t = 1.8$, $P > 0.05$). At the low-elevation site, the differences between diet types were not statistically significant for either the ginger root oil-deprived ($t = 1.2$, $P > 0.05$) or the ginger root oil-exposed ($t = 1.0$, $P > 0.05$) males.

Direct comparisons of the numbers of matings obtained by wild versus *tsl* males (Table 1) mirror the trends described above. At the high-elevation site, the mating success of *tsl* males was low overall, and even the ginger root oil-exposed *tsl* males with enhanced performance were outcompeted by wild males. At the high-elevation site, wild males obtained, on average, significantly more matings per replicate than any treatment type of *tsl* male (protein-fed, ginger root oil-exposed: $t = 2.2$; protein-fed, ginger root oil-deprived: $t = 13.2$; protein-deprived, ginger root oil-exposed: $t = 7.7$; protein-deprived, ginger root oil-deprived: $t = 14.0$; df = 16 and $P < 0.05$ in all tests). As noted above, *tsl* males had higher mating success overall at the lower-elevation site, and here ginger root oil exposure reversed the outcome of mating competition. For the ginger root oil-exposed treat-

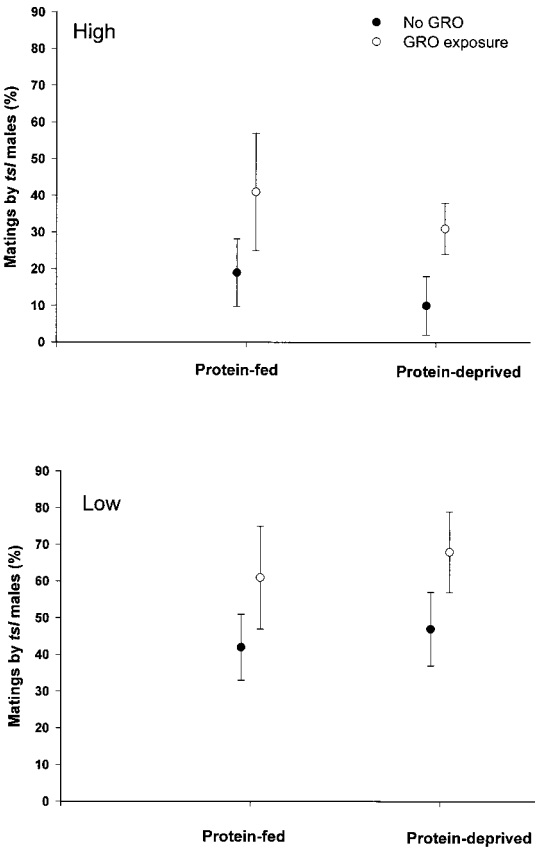

Fig. 2. Relative mating success (% total matings) of *tsl* males in competition with wild males (experiment 1) under different combinations of elevation, ginger root oil (GRO) exposure, and adult diet. Values represent means (±1 SD) of nine replicates.

Table 2. Results of a three-way ANOVA testing effects of elevation, adult diet, and exposure to ginger root oil on the mating performance of *tsl* males (experiment 1). Proportions of matings obtained by *tsl* males in the individual replicates were aresine transformed for the analysis

Source of variation	df	*F*	*P*
Elevation	1	125.6	<0.001
Diet	1	1.326	0.254
Ginger	1	65.702	<0.001
Elevation by diet	1	10.269	0.002
Elevation by ginger	1	1.092	0.300
Diet by ginger	1	0.177	0.675
Elevation by diet by ginger	1	0.0560	0.814
Residual	64		
Total	71		

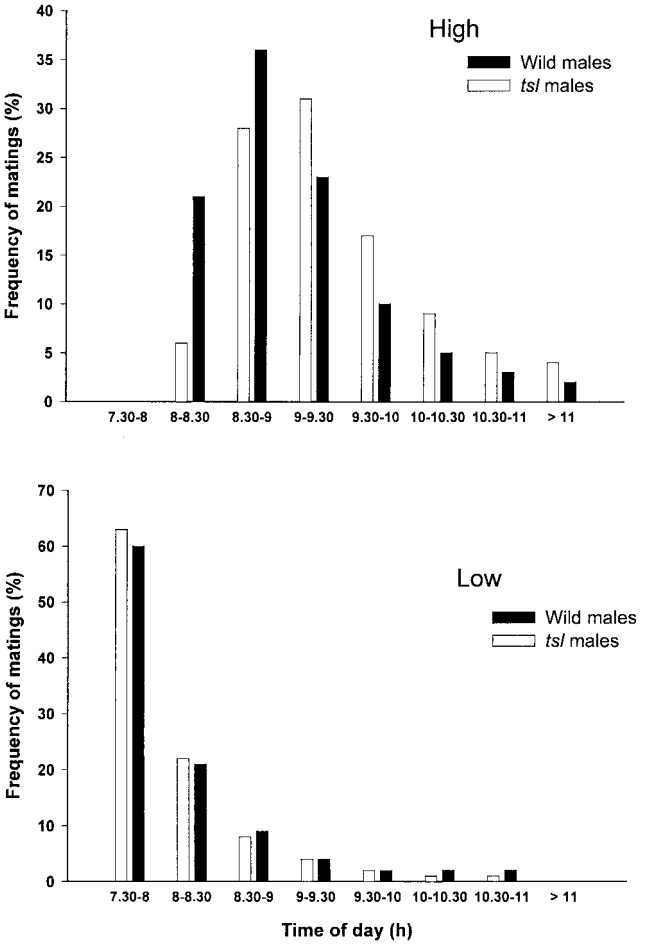

Fig. 3. Frequency distributions of mating start times for wild and *tsl* males at the high- and low-elevation sites, respectively, for experiment 1. Bar height represents the percentage of matings for a given male type that started during the corresponding time period. At the high-elevation site, $n = 1{,}290$ and 490 matings for wild and *tsl* males, respectively. At the low-elevation site, $n = 521$ and 629 matings for wild and *tsl* males, respectively.

ment, both the protein-fed ($t = 3.0$) and the protein-deprived ($t = 5.3$) *tsl* males achieved, on average, significantly more matings than wild males per replicate at the low-elevation site (df = 16 in this and the following tests; $P < 0.001$ in both tests). For the ginger root oil-deprived treatment, however, the protein-fed *tsl* males mated significantly less often than wild males ($t = 2.6$; $P < 0.05$), whereas no difference in mating frequency was detected between protein-deprived *tsl* males and wild males ($t = 1.0$, $P > 0.05$).

To examine possible temporal differences in mating activity, we first compared the distributions of start times of matings involving protein-fed versus protein-deprived *tsl* males within the ginger root oil-exposed or ginger root oil-deprived categories, respectively, at both study sites. These distributions did not vary significantly for either ginger root oil treatment at either site (χ^2 test, $P > 0.05$ in all four tests, df = 5 and 4 at high- and low-elevation sites, respectively). Data were then pooled across diets, and start times were compared between matings involving ginger root oil-exposed or ginger root oil-deprived *tsl* males at both sites. No significant variation was detected between ginger root oil treatments at either the high- ($\chi^2 = 10.4$, $P > 0.05$, df = 6) or low- ($\chi^2 = 9.9$, $P > 0.05$, df = 5) elevation sites.

Based on the preceding findings, data for the *tsl* males were pooled across all treatment categories and compared with those for wild males at each site (Fig. 3). The temporal distribution of start times differed significantly for matings involving wild versus *tsl* males at the high-elevation site ($\chi^2 = 105.5$, df = 7, $P < 0.001$) but not at the low-elevation site ($\chi^2 = 8.5$, df = 6, $P > 0.05$). At the high-elevation site, 20% of matings involving wild males occurred before 0830 hours and 56% occurred before 0900 hours. In contrast, only 6% of matings involving *tsl* males occurred before 0830 hours and only 34% occurred before 0900 hours. Presumably reflecting higher temperatures, most matings started earlier in the morning at the low site, and ≈85%

of matings were initiated before 0830 hours for both wild and *tsl* males. Temporal distributions of mating start times differed between the high- and low-elevation sites for both wild ($\chi^2 = 653.9$, df = 7, $P < 0.001$) and *tsl* ($\chi^2 = 832.0$, df = 6, $P < 0.001$) males.

Because of the aforementioned difference in temperature regimes (Fig. 1), we analyzed data on copulation duration differently for the two sites. Specifically, temporal variation in copulation duration was investigated only at the high-elevation site, where mating start times were distributed more evenly across the morning (Fig. 3). For the high-elevation site, we first compared copulation durations among the four types of *tsl* males. Because the temporal distribution of mating start times did not vary among the treatment categories of *tsl* males (as described above), data were pooled across all hours and analyzed in a two-way ANOVA with diet and ginger root oil exposure as factors. Based on this analysis, copulation duration varied significantly with diet ($F = 20.5$; df = 1, 486; $P < 0.001$) but not with ginger root oil treatment ($F = 0.7$, $P > 0.05$). The diet by ginger root oil interaction was not significant ($F = 2.9$, $P > 0.05$). Based on these results, data were combined across ginger root oil treatments for protein-fed and protein-deprived *tsl* males, respectively, in the following analyses.

Independent of mating start times, significant variation in copulation duration was detected among wild males, protein-fed *tsl* males, and protein-deprived *tsl* males at the high-elevation site ($F = 73.6$; df = 2, 1777; $P < 0.001$), with significant differences apparent in all pairwise comparisons ($P < 0.05$ in all tests, Tukey's test). Mean (±1 SD) copulation durations were 154.4 ± 40.9 min (range, 6–308) for wild males, 119.8 ± 40.4 min (range, 15–225) for protein-fed *tsl* males, and 141.4 ± 40.5 min (range, 11–255) for protein-deprived *tsl* males, respectively (Fig. 4). Copulation duration varied significantly with starting times (grouped by 0.5-h intervals) for both wild ($F = 51.3$; df = 5, 1284; $P < 0.001$) and *tsl* (protein-fed: $F = 11.4$; df = 5, 358; $P < 0.001$; protein-deprived: $F = 4.3$; df = 5, 130; $P < 0.05$) males, with copulations started early in the morning lasting longer, on average, than those initiated later (Fig. 5).

This temporal pattern in copulation duration did not seem to reflect size differences between early- and late-mating flies. We found no significant size difference between males or females from the first and last 15 pairs, respectively, in any of the three cages considered (*t*-test; df = 28 and $P > 0.05$ in all tests).

At the low-elevation site, a two-way ANOVA revealed no clear trends in copulation duration among the different groups of *tsl* males. The diet by ginger root oil treatment interaction term was significant ($F = 38.1$; df = 1, 625; $P < 0.001$): among protein-fed *tsl* males, copulation duration was significantly shorter for ginger root oil-exposed individuals (mean = 101.1 ± 33.4 min) than nonexposed individuals (mean = 126.0 ± 32.2 min; $t = 5.5$; df = 292; $P < 0.001$), whereas among protein-deprived males, copulation duration was longer for ginger root oil-exposed individuals (mean = 129.5 ± 25.5 min) than nonexposed individuals (mean = 118.2 ± 20.3 min, $t = 2.5$, df = 333, $P < 0.05$). Pooling data across treatment groups for the low-elevation site, we found that copulations involving *tsl* males (mean = 118.4 ± 32.2 min) were significantly shorter than those involving wild males (mean = 142.3 ± 36.7 min, $t = 9.5$, df = 1148, $P < 0.001$). Overall, copulations involving wild males were significantly shorter at the low-elevation site than the high-elevation site ($t = 3.9$, df = 1809, $P < 0.001$), whereas there was no significant difference between sites for *tsl* males ($t = 1.3$, df = 1127, $P > 0.05$).

Experiment 2: Competition between *tsl* Males. In the absence of wild males, the ginger root oil-exposed *tsl* males had a pronounced mating advantage over ginger root oil-deprived *tsl* males at both the high- ($t = 7.8$, df = 16, $P < 0.001$) and low- ($t = 6.8$, df = 16, $P < 0.001$) elevation sites (all males were protein-fed). On average, ginger root oil-exposed males accounted for 69% and 73% of the total matings per replicate at the high- and low-elevation sites, respectively. The mean numbers of matings per replicate were as follows: high-elevation site, ginger root oil-exposed = 45.7 ± 7.7, ginger root oil-deprived = 20.0 ± 6.1, and low-elevation site, ginger root oil-exposed 52.7 ± 10.9, ginger root oil-deprived = 20.7 ± 9.0.

In this experiment, temporal distributions of mating start times did not differ between ginger root oil-exposed and ginger root oil-deprived males at either the high- ($\chi^2 = 8.2$, df = 6, $P > 0.05$) or low- ($\chi^2 = 0.8$, df = 5, $P > 0.05$) elevation sites. Pooled data across ginger root oil treatments revealed that, as in experiment 1, the temporal pattern of mating start times differed between sites ($\chi^2 = 304.2$, df = 6, $P > 0.05$), with matings starting earlier in the day at the low-elevation site. However, the absence of wild males in experiment 2 had no obvious effect on the timing of matings by *tsl* males as the distributions of mating start times for *tsl* males were similar between experiments 1 and 2 for both the high- ($\chi^2 = 4.2$, df = 6, $P > 0.05$) or low- ($\chi^2 = 4.8$, df = 5, $P > 0.05$) elevation sites.

Discussion

Perhaps the chief contribution of the current study is the documentation of interhabitat variation in the mating competitiveness of sterile male medflies from the same mass-rearing strain. To our knowledge, the current study provides the first demonstration of this phenomenon for any tephritid species for which SIT is used as a management tool. Field-cage mating tests conducted following the same protocol and using the same host plant (coffee) revealed that *tsl* males had, in general, much higher mating success at 700-m elevation than 1,200-m elevation. The reasons underlying this observation are unknown. Although various environmental parameters, such as relative humidity and light intensity, may have varied with altitude and may have affected mating performance, air temperature was probably the most influential parameter. In experiment 1 at the high-elevation site, wild males (which, as noted above, were derived from collections of high-elevation populations) obtained many matings

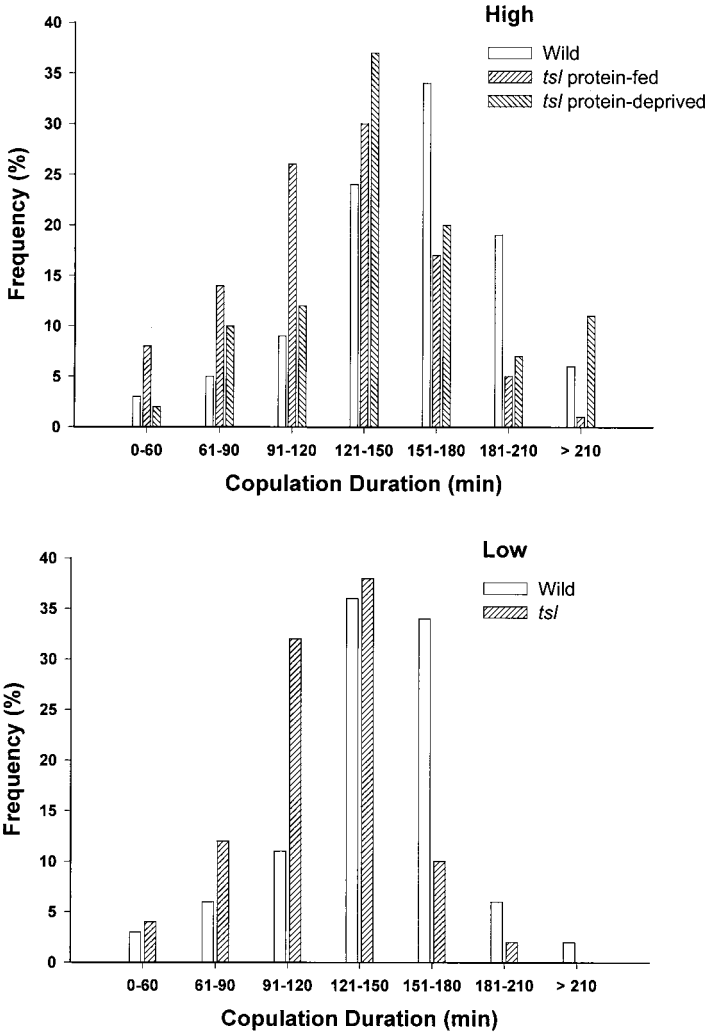

Fig. 4. Frequency distributions of copulation duration for matings involving wild males and *tsl* males at the high- and low-elevation sites. For the high-elevation site, $n = 1{,}290$ for wild males, $n = 364$ for protein-fed *tsl* males, and $n = 126$ for protein-deprived *tsl* males (for *tsl* males, data pooled across ginger root oil treatments for each diet). At the low-elevation site, $n = 521$ and 629 matings for wild and *tsl* males, respectively.

when temperatures were below 19–20°C (before 0900 hours), whereas *tsl* males mated frequently only when temperatures exceeded that level. This same trend could have arisen if *tsl* males were active at the cooler temperatures but discriminated against by the wild females. However, data from experiment 2 indicate that *tsl* males were, in fact, not active at the cooler temperatures, i.e., at the high-elevation site, the distribution of mating start times of *tsl* males in experiment 2 (wild males absent) was similar to that recorded in experiment 1 (wild males present). At the low-elevation site, in contrast, the air temperature, on average, was already ≈22°C at 0730 hours, and *tsl* males mated frequently in the early morning.

The *tsl* strain studied herein has been mass-reared and maintained at relatively high temperatures for over 15 generations. At the El Pino rearing facility, larvae are held at 25–35°C, pupae at 19–21°C, and adults at 23–25°C. This thermal regime imposed over many generations creates strong artificial selection and may limit the effectiveness of released *tsl* males to areas having a similar range of temperature. Thus, in Guatemala, SIT may be more effective in warmer low-elevation areas than cooler mountainous areas. Such information should be considered in the planning and implementation of areawide control in the Mexico-Guatemala region, and alternative or supplemental practices, such as biocontrol through wasp parasitoids, should assume greater importance.

To expand upon this suggestion, the altitudinal variation in the performance of *tsl* males provides a caveat regarding the emerging view that a single generic strain of mass-reared medfly can be used in different SIT programs worldwide (Robinson et al. 2002). Ev-

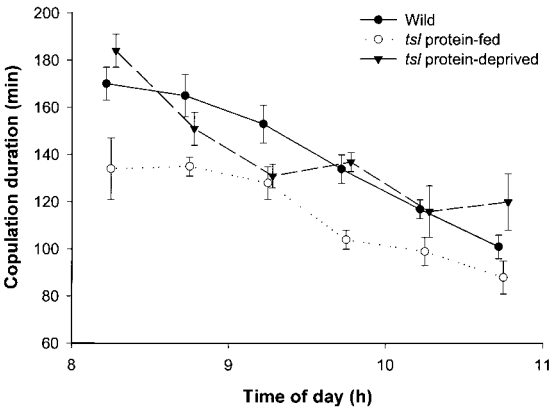

Fig. 5. Relationship between copulation duration and mating start time for matings involving wild and *tsl* males. Symbols represent mean duration (±SE) of copulations initiated during a given 0.5-h interval. For *tsl* males, data were pooled across ginger root oil treatments for each diet type.

idence of limited genetic (Gasparich et al. 1997) and behavioral (Cayol 2000) divergence among wild medfly populations clearly supports the "one strain for all programs" view. However, the apparent absence of premating isolation barriers among wild populations should not be misconstrued as evidence that males of a generic, mass-reared strain will compete uniformly well in all regions in which they are released. Mass production under a narrowly prescribed set of ambient conditions, whether for a generic strain or a regional one, will yield flies strictly adapted to the environmental regime established in the rearing facility. If the relative performance of a mass-reared strain varies with the climatic similarity between the release area and the rearing rooms, then modifying rearing conditions to better mimic the climate of the release areas, or using multiple rearing environments for releases in climatically variable areas, may be a necessary step to guaranteeing full effectiveness of SIT in different regions or habitats.

Independent of elevation, ginger root oil exposure enhanced the mating success of *tsl* males in all tests. Similar procedures of ginger root oil exposure have generated similar results for mass-reared Mediterranean fruit fly males competing against wild males from Hawaii (Shelly and McInnis 2001, McInnis et al. 2002) and Madeira (Shelly et al. 2002a) as well. Also, although additional work is required, initial work (Shelly et al. 2002a) indicates that ginger root oil exposure has no effect on male survivorship. The specific factor(s) underlying enhancement of mating performance is unknown, although ginger root oil exposure was found to increase male signaling (pheromone-calling) activity (Shelly 2001). The heightened mating success of ginger root oil-exposed *tsl* males in the current study did not result from any temporal shift in mating activity as distributions of mating start times were similar between ginger root oil treatments at both study sites. Regardless of the mechanism, more recent work (T.E.S. and D.O.M., unpublished data) has revealed that ginger root oil exposure of *tsl* males in prerelease, eclosion (PARC) boxes (volume = 0.1 m^3; 40,000 males per box) significantly increases mating competitiveness as well, and ongoing work is investigating the possibility of aromatizing entire rooms (containing many PARC boxes) to boost the mating performance of *tsl* males.

Although the limited data available on ginger root oil are fairly consistent, those regarding the influence of diet on male mating success are equivocal for the Mediterranean fruit fly. Kaspi and Yuval (2000) found that, in competition with wild males for wild females, mass-reared males fed a protein-sugar mixture as adults obtained more matings relative to mass-reared males that were fed sugar only. Similarly, Kaspi et al. (2000) studied wild males in field cages and found that protein-fed males signaled and mated more frequently than protein-deprived males. Although no difference in signaling activity was evident, Shelly et al. (2002b) found that aggregations of protein-fed males attracted approximately twice as many females as aggregations of protein-deprived males in field tests. However, additional work in Hawaii (Shelly and Kennelly 2002; T.E.S., unpublished data) has shown that, although the addition of protein to the adult diet enhances mating success in wild males, diet composition has no detectable effect on the mating performance of mass-reared males when competing against wild males. Likewise, the current study found no consistent effect of diet on the mating success of *tsl* males. In general, protein-fed males mated more frequently at the high site and protein-deprived males mated more frequently at the low-elevation site, but most pairwise comparisons within ginger root oil treatment categories at a site were not statistically significant. In sum, it seems that the addition of protein to the adult diet has a variable effect on male mating success and on the efficacy of SIT in turn.

The current study revealed that, at both the high- and low-elevation sites, matings with wild females that involved wild males lasted significantly longer than those involving mass-reared males. In contrast, Orozco and Lopez (1993) and Shelly and Kennelly (2002) found no significant differences in average copulation duration between pairs of wild females and wild versus mass-reared males. Cladera et al. (2001) performed mating trials in which males from different mass-reared strains competed with wild males (for wild females) from the same strain and found significant differences in copulation duration between mass-reared and wild males for certain pairs of strains but not others. Thus, just as natural variation occurs among wild populations (references in Shelly and Kennelly 2002), males of different mass-reared strains seem to vary in the amount of time spent in copula (Field et al. 1999).

In addition to interstrain differences, temperature had a pronounced effect on copulation duration. For both wild and *tsl* males, the average length of copulations declined during the day at the high-elevation site (as temperatures increased), and copulations were, on average, shorter at the low-elevation site

(where temperatures were relatively high) than the high-elevation site. Among *tsl* males, the effects of diet and ginger root oil exposure were less clear-cut. At the low-elevation site, these factors interacted but in an inconsistent manner, i.e., ginger root oil exposure coupled with the protein-less diet resulted in relatively long copulations, whereas ginger root oil exposure coupled with the protein-containing diet resulted in relatively short copulations. In contrast, at the high site, ginger root oil exposure had no apparent effect, whereas matings with protein-deprived males lasted longer, on average, than those with protein-fed males. Field and Yuval (1999) report a similar finding for a laboratory-reared strain in which protein-deprived males had low mating competitiveness and lessened ability to inhibit female remating (Blay and Yuval 1997). Given these constraints, protein-deprived males may benefit from prolonged copulation by sequestering the female from rival males and increasing the amount of sperm transfer, thus maximizing his advantage in future sperm competition (Field and Yuval 1999). Although the present findings offer some support for this notion, copulation duration is most likely the outcome of a complex interaction of factors, including abiotic conditions, predation risk, ages, sizes, nutritional states, and mating experiences of the mating pair, and the ovipositional experience of the female (Field et al. 1999, Field and Yuval 1999), and assessment of their relative importance awaits a comprehensive experimental study under controlled conditions.

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